

Immunomodulation of Cell-Mediated Cytotoxicity after Chronic Exposure to Vapors

Aleksandar Bulog¹, Irena Brčić Karačonji², Ivana Šutić³ and Vladimir Mićović¹

¹ Department of Public Health, School of Medicine, University of Rijeka, Rijeka, Croatia

² Institute for Medical Research and Occupational Health, Zagreb, Croatia

³ School of Medicine, University of Rijeka, Rijeka, Croatia

ABSTRACT

Cytotoxic T lymphocytes and natural killer (NK) cells represent two major types of immune cytotoxic cells that have a crucial role in innate immunity. Urban air pollution represents a common and difficult problem in the majority of metropolises, which contain high levels of traffic congestion generating great amounts of genotoxic substances. Diesel exhaust particles act as adjuvant in the immune response and may lead to the enhancement of proinflammatory and proallergic response without exposure to allergen. The activities of diesel exhaust particles (DEPs) and their benzene extracts may cause the increasing respiratory mortality and morbidity. Since the lung inflammatory response to DEPs is compartmentalized, the importance of finding the suppressive substances, whose function will be to elucidate the DEPs effects, is fundamental. The objectives of this study were to examine the NK-mediated cytotoxicity against human NK-sensitive target, as well as the values of BTEX (benzene, toluene, ethylbenzene and xylene isomers) in urine in people from industrial area and those from rural fields. NK-mediated cytotoxicity of peripheral blood mononuclear cells was investigated against human NK-sensitive K-562 tumor cell line. BTEX were analyzed from urine by a HS-SPME method. All exposed individuals were demonstrated the significant decreased of NK-mediated cytotoxicity against human NK-sensitive target and increasing BTEX values. BTEX-analyze in urine and determination of NK-mediated cytotoxicity, as an important methods represent good markers in environmental monitoring, which contribute to the better protection of air pollutants and quicker diagnosis of environmental diseases.

Key words: BTEX, environmental diseases, innate immunity, NK-mediated cytotoxicity

Introduction

Volatile aromatic hydrocarbons benzene, toluene, ethylbenzene and xylene isomers (o-, m-, p-) (BTEX) are organic compounds that are widely used as very strong organic solvents, and appear as a component of paints and varnishes used in protective coatings of various metal and wood surfaces. Because of its physico-chemical properties (colorless liquid, low boiling point, characteristic odor, a high vapor pressure, high possibility of evaporation) they can easily be found in the environment and people are more or less constantly exposed to their action, especially employees who coated the ship hull, and the nature of their work are exposed through inhalation of volatile compounds and components of direct contact with the color. BTEX compounds are found in gasoline, and are discharged into the atmosphere through the exhaust of cars and emissions from large industrial plants

such as refineries for processing oil products. Toxic effects of BTEX were not equal for all compounds, most data were collected for benzene because of its hematotoxicity and carcinogenicity, followed by toluene because of its neurotoxicity, while for the ethylbenzene and xylene isomers there are very poor evidence. All compounds act as an organic solvent locally on the skin and mucous membranes, and after resorption it is seen their systematic effect on the central nervous system. Benzene has very strong hematotoxic action reducing the number of blood cells, as well as bone marrow, accompanied with hypoplasia, pancytopenia, and the end result may be the development of acute or chronic myeloid leukemia. Benzene may cause very strong inhibition of incorporation of iron in red blood cells which is very harmful in the early development of the human organism. Occupational expo-

sure of humans to benzene is very dangerous to health¹⁻⁴. It has been proven that occupational exposure to benzene can lead to chromosomal aberrations, sister chromatid exchange, micronuclei and the occurrence of inhibition of synthesis of DNA and RNA molecules since benzene and its metabolites form DNA adducts⁵⁻⁷. Neurotoxic effects of toluene and xylene in humans are caused by their presence in the phospholipid cell membranes of the nerve and disruption of its essential role (excitability in the creation of an action potential) and the presence of toluene and xylene in the myelin of nerve fibers, which can disrupt their role in the conduction of electrical impulses⁸. Symptoms of neurotoxic effects in humans is manifested as severe headache, dizziness, drowsiness and sometimes loss of consciousness, which can result in acute poisoning if the residence time in this work environment is not curtailed to a minimum. For ethylbenzene, the data are very poor, but there are studies that prove its acute and chronic toxicity of the central nervous system which may manifest in the form of severe headaches, insomnia, irritability and fatigue^{9, 10}. Many immune cells are involved in a chain reaction of the human immune system that are triggered by environmental contaminants. T lymphocytes are usually divided into two basic groups, CD4+ and CD8+ cells and can be triggered by various environmental antigens¹¹. CD8+ T cells that can slow down the immune response and may develop into cytotoxic T cells, kill target cells that are exposed to polycyclic aromatic hydrocarbons (PAHs), volatile aromatic hydrocarbons (BTEX), diesel exhaust particles (DEP) and heavy metals (Hg, Cd, Pb)¹²⁻¹⁵. The people who are occupationally exposed to increasing concentrations of toluene, and mixtures of toluene and benzene, demonstrated a statistically significant decrease in the total number of subpopulation of NK cells, cytotoxic CD8+ T lymphocytes, helper CD4+ T lymphocytes, B cells, while for the changes at the level of cytotoxic CD8+ T lymphocytes and NK cells, exist some different data¹⁶⁻¹⁸.

Subjects and Methods

In this experiment we included a group of subjects of Rijeka (n=35) who lives and works in a typical metropolitan area (urban area), the group of respondents who live and work on the island of Mali Lošinj (n=35) as characteristic of unpolluted areas (rural) and occupationally exposed subjects who worked on marine construction in the shipyard »Viktor Lenac« (n=35) and this same group of respondents who work on marine construction at the shipyard »Kraljevica« (n=28). Occupationally exposed groups of participants were daily coating of marine construction (shipbuilding) and are exposed to volatile aromatic hydrocarbons benzene, toluene, ethylbenzene and xylene isomers through inhalation and contact, using paints, varnishes, solvents, thinners and biocidal products which have volatile aromatic hydrocarbons as organic solvent. NK-mediated cytotoxicity of peripheral blood mononuclear cells was investigated against human

NK-sensitive K-562 tumor cell line. BTEX were analyzed from urine by a HS-SPME method. The results were analyzed using the Sigma Plot for Windows, version 1.02. Statistical analyses were performed using a Student, s t-test one way analysis for comparison of means. All experiments have been approved by a local ethics committee.

Results

We examined and analyzed the changes in BTEX in urine of workers and the shipyard Viktor Lenac Kraljevica, and residents of urban areas of Rivers and rural areas of the island of Mali Lošinj. If we compare the concentrations of BTEX in population of Rijeka and Mali Lošinj significantly lower concentrations of BTEX have residents of rural areas of island of Mali Lošinj. Workers in shipyards have significantly increased the value of BTEX in urine compared with the inhabitants of the urban and rural areas (Figure 1).

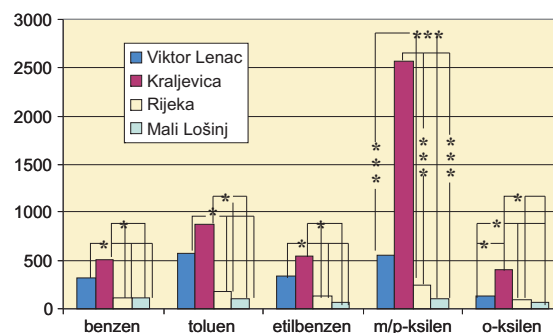


Fig. 1. Summary result for the average values of the concentration of BTEX in urine in all investigated groups.

Analysis of changes in the NK-cytotoxicity test

NK-activity measured spontaneous cytolytic activity of natural killer or NK cells. In contrary to cytotoxic T lymphocytes, which need several days for the development of killer activity, NK cells are effective within a few hours of incubation with target cells. Target cells (peripheral blood lymphocytes) of inhabitants living near gasoline industry and controls, are mixed together in certain proportions and incubating over 2 hours, after which we measured cytotoxic activity, using flow cytometry. The results of our study show that the cytolytic activity of NK cells of inhabitants living near gasoline industry against human tumor K-562 line, is significantly lower ($p < 0.05\%$) compared to the cytolytic activity of NK cells of control subjects (Figure 2).

Discussion and Conclusion

Urban air pollutants are known to contribute to increased prevalence of environmental diseases and multiple toxic chemical sensitivity or other related syndromes, as a result of an abnormal immune response based on en-

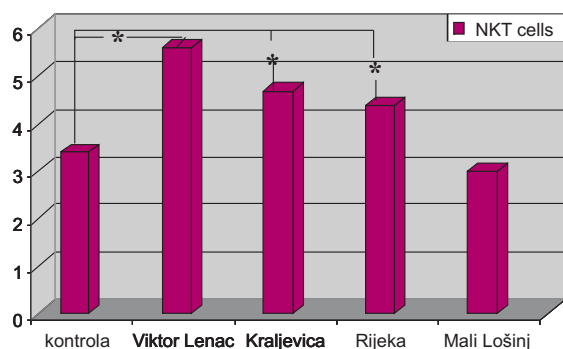


Fig. 2. Reduced cytotoxic activity of NK cells of inhabitants living near oil industry in relation to the control group at 2 h test cytotoxicity against NK-sensitive human K-562 cells.

environmental damage of lymphocyte subsets¹⁶. Besides the characteristics of the human organism (physiological, biochemical, genetic), and chemical characteristics of potentially hazardous pollutants (solubility, volatility), many other environmental factors affect the incidence of the environmental diseases. Factors such as temperature, humidity and light intensity, have a major impact on the biological processes of accumulation and bioavailability of contaminants in the body. Bioavailability is the degree to which pollutant is free to enter into the body

and potentially produce effects at the site of action, and these places are the site of entry (locally), output area (systematic), and a place of secretion (skin, visible mucous membranes). Toxic environmental factors which have a possibility to induce immunological changes may contribute to develop environmental diseases. Between all of these environmental factors, the concentration of pollutants in the environment itself however is a key reason for appearance of certain diseases of the environment¹⁸. Volatile aromatic hydrocarbons benzene, toluene, ethylbenzene, o-xylene and m/p-xylene have been proven in higher concentrations in those areas with developed oil industry and increased traffic frequency. Our results have showed that the concentrations of BTEX in urine are increased in shipyard workers in relation to residents of urban areas (Rijeka), and in particular the major changes compared with the inhabitants of rural areas (Mali Lošinj). People from industrial area have significantly increased BTEX (benzene, toluene) in urine, which correlate with decreased cytotoxic potentials of peripheral lymphocytes.

Acknowledgements

This work was supported by grants from the Croatian Ministry of Science (No 0621341-0308 and 0620096-0094).

REFERENCES

1. PEKARI K, *Natural and Environmental Sciences*, 16 (1994) — 2. BOGADI-ŠARE A, TURK R, KARAIĆIĆ V, ZAVALIĆ M, TRUTIN-OSTOVIĆ K, *Toxicol Ind Health*, 13 (1997) 458. — 3. MAITRE A, SOULAT JM, MASCIET P, STOKLOV M, MARQUES-GAUDEMARI R, *Scand J Work Environ Health*, 28 (2002) 402. — 4. RUCHIRAWAT M, NAVASUMRIT P, SETTACHAN D, AUTRUP H, *Annals New York Academic of Sciences*, 1076 (2006) 678. — 5. KOLACHANA P, SUBRAHMANYAM VV, MEYER KB, ZHANG L, SMITH MT, *Cancer Research*, 53 (1993) 1023. — 6. SØRENSEN M, AUTRUP H, HERTEL O, WALLIN H, KNUDSEN LE, LOFT S, *Cancer Epidemiol Biomarkers Prev*, 12 (2003) 191. — 7. SØRENSEN M, SKOV H, AUTRUP H, HERTEL O, LOFT S, *Science of the total environment*, 309 (2003) 69. — 8. BONO R, SCURSATONE E, SCHILIRO T, GILLI G, *Journal of Toxicology and Environmental Health*, 66 (2003) 519. — 9. APPLGATE BM, KEHRMEYER SR, SAYLER GS, *Appl Environ Microbiol*, 64 (1998) 2730. — 10. YUEH-FEN L, FENG-YIN L, CHEN-LUNG H, VIVIAN HSIU-CHUAN L, *Environmental Pollution*, (2007) 1. — 11. BENTLEY AM, MENZ G, STORZ C, ROBINSON DS, BRADLEY B, JEFFERY PK, DURHAM SR, KAY AB, *Am Rev Respir Dis*, 146 (1992) 500. — 12. AZZAWI M, JOHNSTON PW, MAJUMDAR S, KAY AB, JEFFERY PK, *Am Rev Respir Dis*, 145 (1992) 1477. — 13. KEMENY DM, *Toxicology*, 153 (2000) 3. — 14. PEDEN DB, *Environ Health Perspect*, 108 (2000) 475. — 15. BUSSE W W, ROSEN FS, *New Eng J Med*, 344 (2001) 350. — 16. MIČOVIĆ V, VOJNKOVIĆ B, BULOĞ A, COKLO M, MALATESTINIĆ D, MRAKOVCIĆ-SUTIĆ I, *Coll Antropol*, 33 (2009) 743. — 17. LANDIS WG, MING-HO Y, USA: Lewis Publishers (1998) 15. — 18. WALKER CH, HOPKIN SP, SIBLEY RM, PEAKALL DB, USA: Taylor & Francis (2001) 10.

V. Mićović

Teaching Institute of Public Health of Primorsko-goranska County, Krešimirova 52a, 51000 Rijeka, Croatia
e-mail: ravnatelj@zzjzpgz.hr

IMUNOMODULACIJA STANICAMA POSREDOVANE CITOTOKSIČNOSTI NAKON KRONIČNE IZLOŽENOSTI PARAMA

SAŽETAK

Citotoksični T limfociti i stanice prirodne ubojice (NK stanice) predstavljaju dvije glavne vrste citotoksičnih stanica imunološkog sustava koje imaju ključnu ulogu u urođenoj imunosti. Zagađivanje zraka predstavlja težak problem u

većini svjetskih metropola, zbog visoke razine genotoksičnih tvari. Ispušne čestice djeluju kao dodatni pokretači imunološkog odgovora, s ciljem bržeg poticanja proupalnog i alergijskog odgovora bez izlaganja alergenu. Pod djelovanjem raznih ispušnih plinova i derivata benzena može se povećati incidencija respiracijskih poremećaja. Ciljevi ovog istraživanja bili su ispitati NK posredovanu citotoksičnost, kao i vrijednosti BTEX (benzen, toluen, etilbenzen i ksilen izomeri) u urinu osoba koje stanuju u blizini industrijskih postrojenja, osoba profesionalno izloženih hlapljivim aromatskim spojevima, kao i onih iz ruralnih područja. NK-posredovana citotoksičnost mononuklearnih stanica periferne krvi ispitana je u dvosatnom testu citotoksičnosti protiv humane NK osjetljive K-562 tumorske stanične linije. BTEXi su analizirani iz urina HS-SPME metodom. Svi izloženi pojedinci su pokazali značajan pad NK posredovane citotoksičnosti i povećanje BTEX vrijednosti. Ove metode (analiza BTEX-a u mokraći i određivanje NK posredovane citotoksičnosti) predstavljaju dobar način praćenja bolesti okoliša, te mogu pridonijeti boljoj zaštiti od djelovanja onečišćenog zraka i bržoj dijagnostici bolesti okoliša.